

Mini protocol:

1. Dilute all oligos to 100 uM, then 10 uM
 - a. For given nmol, add $(10 \times \text{nmol})$ uL of ddH₂O.
 - b. Then dilute 1:10.
2. Set up the PCR mastermix (x4):
 - a. 96 uL H₂O
 - b. 13.2 uL Expand buffer
 - c. 13.2 uL dNTPs (2mM)
 - d. 2 uL Expand polymerase
 - e. Mix well...
3. To three clean PCR tubes labeled 1A,1B,1C, with name, add:

Tube	Mastermix	Oligo A	Oligo B	Template
1A	31.1 uL	1 uL OJIMH011 (10 uM)	1 uL OJIMH013 (10 uM)	0.5 uL Bca1623 6-9
1B	31.1 uL	1 uL OJIMH012 (10 uM)	1 uL CA1674 (10 uM)	0.5 uL Bca1559 7-7
1C	31.1 uL	1 uL CA1675 (10 uM)	1 uL OJIMH014 (10 uM)	0.5 uL Bca1559 7-1

4. Use the 2K55 temperature program (1+ hour)
5. Retrieve DNA, throw away dilute oligos, STORE original oligos and DNA in freezer.